

# Cell counting with Neubauer Chamber

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## Materials

- Cellular dilution to be measured

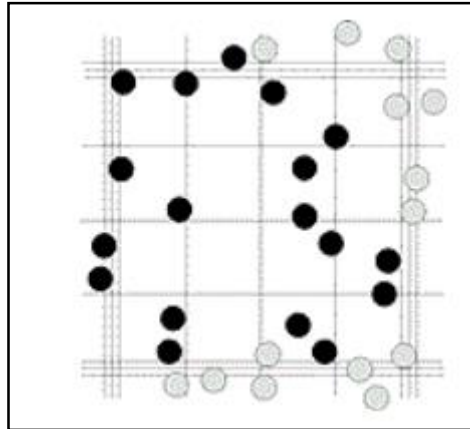
## Apparatus

- Neubauer chamber
- Glass cover
- Optical microscope
- Micropipette and disposable tips

## Methods

1. Prepare a dilution, if necessary, until the recommended concentration:  $10^6$  cells/mL (1 million cells/mL);
2. Put the glass cover on the Neubauer chamber central area. Use a flat surface to place the chamber;
3. Adjust the micropipette to suck 10  $\mu$ L;
4. Introduce the micropipette tip on the dilution previously prepared;
5. Push the pipette plunger slowly;
6. Place pipette tip close to the glass cover edge, right at the center of the Neubauer chamber;
7. Release the plunger slowly observing how the liquid enters the chamber uniformly, being absorbed by capillarity;
8. In case of the appearance of bubbles, or that the glass cover has moved, repeat the operation;
9. Place the Neubauer chamber on the microscope stage;
10. Turn on the microscope light;
11. Focus the microscope until you can see a sharp image of the cells looking through the eyepiece and adjusting the stage;
12. Look for the first counting grid square where the cell count will start;

13. Start counting the cells in the first big square (the chamber has nine big squares). Cells touching the upper and left limits should be counted, unlike cells touching the lower and right limits which should not be taken into account.



14. Apply the formula for the calculation of concentration:

$$\text{Concentration} = \frac{\text{Number of cells}}{\text{Volume (in mL)}}$$

The number of cells will be the sum of all the counted cells in all squares counted. The volume will be the total volume of all the squares counted. Since the volume of 1 big square is 0.1  $\mu\text{L}$ , the formula used when counting big squares is:

$$\text{Concentration} = \frac{\text{Number of cells} \times 10,000}{\text{Number of big squares}}$$

In case a given dilution was applied, the concentration obtained should be converted to the original concentration before the dilution.